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SKIN TIGHTENING AGENT
[Hifu hikishimezai]

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(54) [Title of the Invention] /*

Skin Tightening Agent

[Claim(s)]

[Claim 1] An agent for ameliorating sagging and tightening of skin comprising an integrin expression promoting agent for human skin cells as an active ingredient.

[Claim 2] The agent for ameliorating sagging and tightening of the skin in Claim 1, wherein the integrin is integrin α2β1.

[Claim 3] The agent for ameliorating sagging and tightening of the skin of Claim 1 or 2, wherein the human skin cells are human skin fibroblasts.

[Claim 4] The agent for ameliorating sagging and tightening of the skin of any one of Claims 1 to 3, wherein the integrin expression promoting substance is one or more plants selected from a group comprising burdock root, rosemary, kiwi, Pachyma hoelen, carrot/ginseng, and red algae, or extracts thereof.

[Detailed Specifications]

[0001] [Technical Field of the Invention]

The present invention relates to a composition which ameliorates the sagging of skin and provides a tightened feeling.

[0002] [Prior Art and Problems to be Solved by the Invention]

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*Claim and paragraph numbers correspond to those in the foreign text.

The skin is constantly being exposed to external irritations by drying, UV rays, and such, and along with aging, tightness and elasticity are lost, and sagging occurs.

[0003] Agents for ameliorating this sagging and tightening of the skin have not existed conventionally. Although it has been known that astringents have skin tightening effects, the action of an astringent merely reduced blood serum temporarily and had a vasoconstrictive action, and did not ameliorate sagging of the skin.

[0004] Consequently, an object of the present invention is to provide an agent for ameliorating sagging and tightening of the skin having effects on sagging of skin and loss of tightness.

[0005] [Means for Solving the Problems]

In view of such actual circumstances, the present invention focused on the interaction between skin cells, and in particular, skin fibroblasts and the extracellular matrix, such as collagen.

[0006] That is, the skin is divided mainly into three layers including the epidermis, dermis, and hypodermis. Of these layers, the dermis is extremely important for maintaining the structure of the skin, and is composed of rigid and soft fibers including collagen, fibronectin, and elastin that form the connective tissue for the dermis. Human skin cells, and in particular, human skin fibroblasts interact with fibers, such as collagen, fibronectin, and elastin, whereby the state of the connective tissues is controlled. As a consequence, it is thought that the interaction between the skin

fibroblasts and the extracellular matrix, such as collagen, is deeply related to preserving or losing skin tightness.

[0007] On the other hand, the cells adhere to the extracellular matrix via integrin, which is a specific receptor expressed on the cell surface. The importance of this integrin has increased in recent years, but its application for suppressing the adhesion of white blood cells, inhibiting the agglutination of platelets, suppressing metastasis, or treating and preventing heart attacks, arteriosclerosis, osteolytic diseases, and the like has merely been studied.

[0008] Therefore, as a result of further painstaking research, the inventors of the present invention focused on integrin, which had not been noticed at all thus far in skin cells, and discovered that by increasing the amount of this integrin, the interaction between skin cells, and in particular, skin fibroblasts and the extracellular matrix could be activated, the sagging of skin could be ameliorated, tightness could be maintained and recovered, and the skin could be tightened, which led them to perfecting the present invention.

[0009] That is, the present invention provides an agent for ameliorating sagging and tightening of the skin composed of an integrin expression promoting substance for human skin cells as the active ingredient.

[0010] [Embodiments of the Invention]

The integrin in the present invention should be expressed in human skin cells, but it is preferably expressed in human fibroblasts. When it is expressed in human skin fibroblasts, an outstanding skin tightening effect can be manifested. Integrin comprises α subunits and β subunits. The α subunits further include α1 to α5, αL, and so forth, and the β subunits include β1, β2, β3, etc. But when the interactions between the matrices, such as collagen, vitronectin, fibronectin, and laminin, and the cells present in connective tissue, such as fibroblasts, are considered, the expression of the α2 subunit, α5 subunit, and β1 subunit thereamong is preferably promoted, and the expression of the α2 subunit is further promoted, while it is more desirable that the expression of the β1 subunit be simultaneously promoted. In addition, in relation to skin fibroblasts, the expression of the α2β1 integrin is preferably promoted from the viewpoint of the integration with collagen.

[0011] As long as it promotes the expression of the integrin of human skin cells, the active ingredient of the agent for ameliorating sagging and tightening of the skin of the present invention can be any agent, but it is preferably one or more plants selected from a group comprising burdock root, rosemary, kiwi, Pachyma hoelen, carrot/ginseng, and red algae, or extracts thereof.

[0012] These plants or extracts thereof are already generally known as external skin preparations, cosmetics, raw materials for medicinal products, base materials, and additives. Moreover, it is

also known that they have effects, such as humidifying effects, anti-inflammatory, blood circulation promoting, hair tonic, and skin beautifying effects. However, being able to tighten the skin by promoting the expression of integrin of skin cells thereof has been completely unknown.

[0013] "Plants" herein mean whole plants thereof, or one or more of their leaves, leaf stalks, stems, roots, and seeds (called "base" hereinafter), or the dried and crushed products thereof. In addition, the term "plant extracts" means a solvent-extracted liquid, a diluted or concentrated liquid thereof, or a dried powder thereof, each obtained by drying the base or crushing the base without drying it, and subsequently extracting this with a solvent under ordinary temperature or heating, or extracting it using extraction equipment.

[0014] Although water, organic solvents, and mixtures of these are cited for the solvent used in the extraction, an organic solvent, or a mixture of water and an organic solvent is especially preferable. A hydrocarbon, halogenated hydrocarbon, ester, and alcohol are cited for the organic solvent, but ethanol, propanol, propylene glycol, butylene glycol are especially preferable.

[0015] Extraction from the base is performed, for example, as follows. That is, the solvent is added to the base itself, or to a dried product or dried and crushed product thereof, and extracted for 0.5 to 30 days, and preferably, 1 to 15 days at 1 to 100°C, and preferably, 3 to 70°C. Next, the plant extract can be obtained by

suitably filtering the resultant liquid extract, letting it stand, then filtering it, etc. The concerned extract may be diluted, concentrated, or freeze-dried, subsequently prepared as a powder or like a paste, and suitably formulated. Moreover, a purification treatment can be performed, such as deodorization and decoloration, as needed. A plant extract that was extracted in this way can be used, or a commercially-available product can be used.

[0016] The aforesaid plants or their extracts may be used as is as agents for ameliorating sagging and tightening of the skin, but they can be used by suitably formulating them.

[0017] The content of the aforesaid plants or their extracts in the agent for ameliorating sagging of the skin and tightening of the skin of the present invention normally is preferably 0.00001 to 10% by weight, and in particular preferably 0.0001 to 3% by weight as a dried solid content of the effective ingredient, from the viewpoints of the effects, the compoundability, and the feeling in use.

[0018] In addition to the aforesaid plants or their extracts, external base materials that are normally used, and other pharmaceutically effective ingredients may be compounded with the agent for ameliorating sagging of the skin and tightening the skin comprising an integrin expression promoting agent for skin cells of the present invention. The external base used herein may be one based on an oily base, one based on an oil-in-water or water-in-oil type emulsion base, or one based on water. The oily base is not limited in

particular, and a vegetable oil, animal oil, synthetic oil, silicone oil, fatty acid, natural or synthetic glyceride, and the like are cited as examples. Moreover, humectants, UV absorbers, alcohols, chelates, pH modifiers, preservatives, thickeners, colorants, fragrances, and the like may be optionally combined and compounded. Moreover, the above-mentioned pharmaceutically effective ingredients are not limited in particular, and analgesics, anti-inflammatory agents, antimicrobials, vitamins, skin softening agents, and the like may be suitably used, as needed. Ointments, creams, milky lotions, face lotions, gels, packs, poultices, foundations, and the like are cited as forms of external skin preparations.

[0019] The agent for ameliorating sagging of the skin and tightening of the skin of the present invention can be administered in any external or internal method, but external administration is preferred, and administration as an external skin preparation is especially preferred.

[0020] Moreover, there are various methods for detecting an integrin. Flow cytometry (FACScan), immunoblotting, Western blotting, antibody staining, and the like are cited as examples of methods using antibodies, while PCR, Northern blotting, and the like are cited as examples of methods using mRNA. Although skin fibroblasts present in the skin's hypodermis are actually the most preferable for the cells used in the detection of an integrin, fibroblasts in other

tissues, such as lung fibroblasts, may be included therefor, and moreover, chondrocytes or the like may be included therefor.

[0021] [Practical Examples]

The present invention is described in detail next by citing practical examples, but it is not intended that the present invention be restricted by these practical examples.

[0022] <Practical Examples 1 to 7>

1 kg of a dried product of the site of a plant shown in Table 1 was steeped in 5 liters of an extraction solvent for 1 week at room temperature, and the solvent-soluble ingredients were extracted. A total of 10 liters of liquid extract were obtained by repeating the same operation on the residue obtained by separating the liquid extract. The solvent of this liquid extract was distilled off, dried solid under reduced pressure, and the extract was obtained. Moreover, hereinafter, W denotes water; BG denotes 1,3-butylene glycol; and ET denotes ethanol.

[0023] [Table 1]

	[illegible]	[illegible]	[illegible]	[illegible] (g)
[illegible] 1	ローズマリー	全草	50%ET/W	20
[illegible] 2	ローズマリー	全草	80%ET/W	36
[illegible] 3	キウイ	果皮、葉	BG	10
[illegible] 4	ブクリョウ	[illegible]菌核	W	56
[illegible] 5	コウジン	全草	W	36
[illegible] 6	ゴボウ	種子は含まず	W	46
[illegible] 7	ニンジン	[illegible]根	80%ET/W	20

Key for Table 1 [translator's note: see original description for symbols, numbers and English]:

	Plant	Extraction Site	Extraction Solvent	Amount Extracted (g)
Practical Example 1	Rosemary	Whole plant		
Practical Example 2	Rosemary	Whole plant		
Practical Example 3	Kiwi	Fruit, leaves		
Practical Example 4	Pachyma hoelen	Leaf stalks		
Practical Example 5	Red algae	Whole algae		
Practical Example 6	Burdock root	Root or whole plant		
Practical Example 7	Carrot/ginseng	Root		

[0024] <Test Example 1>

Measurement of the measurement activity of the integrin-increasing activity was performed in accordance with the method by Riikonen, et al (J. Biol. Chem. 270, (1995):13548). A 90 mm culture dish (5% fetal calf serum (FCS)-containing DMEM (by Gibco) was inoculated with human skin fibroblasts (derived from human foreskin), and after 24 hours, the plant extracts obtained in Practical Examples 1 and 2, and 4 to 7 were added and cultured to a final concentration of 0.01 to 0.001% by weight (% by weight based on dried solid content). Moreover, a solvent used as the control was added and this was cultured. After 48 hours, trypsin/EDTA was acted on the cells, the cells were peeled off, the trypsin was neutralized with FCS and centrifuged, and the cells were cleaned by discarding the supernatant or such. The cells were washed twice in the same manner with 0.1% FCS and 0.02% NaN_3-containing PBS, after which antihuman integrin α2

antibodies (mouse, made by Gibco Co.), antihuman integrin β1 antibodies (mouse, made by Gibco Co.) and antihuman integrin α2β1 antibodies (mouse, made by Chemicon Corp.) were acted on the cells for 30 minutes at 4°C at concentrations of 1/100 to 1/200, respectively. After the second washing, FITC-labeled mouse IgG1 antibodies were acted as secondary antibodies on the cells at a concentration of 1/100 for 30 minutes at 4°C, after which washing was repeated a three times, and the cells were subsequently analyzed using a FACScan (made by Becton Dickinson). Mouse IgG1 (1 μg/mL) was used as the primary antibody blank for the FACScan. The blank portion was subtracted according to respective fluorescent intensity, and the relative fluorescent intensity was calculated based on a 100% control. The results are shown in Table 2.

[0025] [Table 2]

サンプル	濃縮重量固形分重量%	インテグリンα2絶対値	インテグリンβ1絶対値	インテグリンα2β1絶対値
ローズマリー	0.01	114	138	127
ローズマリー	0.008	108	135	118
クラリュウ	0.01	109	137	119
コウソウ	0.01	126	118	130
ゴボウ	0.01	118	137	133
ニンジン	0.01	104	133	108

Key [translator's note: see original description for symbols, numbers and English]:

Sample	Concentrated Weight Solid Residue (% by weight)	Absolute Integrin α2 Value	Absolute Integrin β1 Value	Absolute Integrin α2β1 Value
Rosemary				
Rosemary				
Pachyma hoelen				
Red algae				
Burdock root				
Carrot/ginseng				

[0026] According to Table 2, an increase in the amount of integrin, and in particular, an increase in the amount of the α2β1 integrin is recognized.

[0027] <Test Example 2>

The skin of panelists using rosemary extract or burdock root extract-compounded gel

<Elasticity evaluation>

A compounded gel with the rosemary extract obtained in Practical Example 1 or the burdock root extract obtained in Practical Example 6 (gels B and C, see Table 3) were used to perform an evaluation on each sample by ten panelists. The respective extract-compounded gels were coated on the insides of the upper arms once a day in the morning and at night for 2 weeks, and the skin elasticity was measured before coating and after 2 weeks using a cutometer SEM 575 (made by Courage and Khazaka Electronic GmbH; suction pressure: 500 mbar; section time: 8 sec; release: 2 sec). The measurement time based on the day-to-day fluctuations was consolidated into a fixed time in the morning, respectively, of each person. Moreover, a gel with no extract compounded (gel A, see Table 3) was used as the control. The average and the standard deviation thereof are shown in Table 4.

[0028] Table 3

	ジェルA	ジェルB	ジェルC
アルギン酸ナトリウム	1．0	1．0	1．0
86%グリセリン	5．0	5．0	5．0
ポリオキシエチレン硬化ヒマシ油（40E.O.）	0．5	0．5	0．5
ローズマリー抽出物	--	0．01	--
ゴボウ抽出物	--	--	0．003
精製水	バランス	バランス	バランス
計	100．0	100．0	100．0

Key [translator's note: see original description for symbols, numbers and English]:

	Gel A	Gel B	Gel C
Sodium alginate 86% Glycerin Polyoxyethylene hardened castor oil (40 E.O.) Rosemary extract Burdock root extract Purified water	Balance	Balance	Balance
Total			

[0029] [Table 4]

		皮膚弾性値	
ジェル	配合エキス（固形分含有量%）	使用前	2週間使用後
ジェルA	無し（コントロール）	0．20（0．033）	0．21（0．036）
ジェルB	ローズマリー（0．01%）	0．21（0．035）	0．232（0．052）
ジェルC	ゴボウ（0．003%）	0．206（0．04）	0．228（0．045）

Key [translator's note: see original description for symbols, numbers and English]:

		Skin Elasticity	
Gel	Compounded extract (solid content residue% by content)	Before use	After using 2 weeks
Gel A	None (control)		
Gel B	Rosemary (0.01%)		
Gel C	Burdock root (0.003%)		

The standard deviation is shown in the parentheses, and the same in the tables hereinafter.

[0030] When the gels B and C were used, according to Table 4, it was confirmed that the skin elasticity improved.

[0031] <Test Example 3> Evaluation of skin tightening degree

Gels A to C in Test Example 2 were used to perform an evaluation on the skin tightening degree of each gel according to ten panelists. Each gel was coated on the insides of the upper arms and the part between the cheek surface and the chin once a day in the morning and at night for 2 weeks, and the feeling of tightening was evaluated according to the following criteria before the application and 2 weeks later. The average and standard deviation thereof are shown in Table 5.

(Criteria)

-1: worsened

0: no change, not seen

1: tightened dimly

2: fairly tightened

3: tightened

4: tightened exceedingly

[0032] [Table 5]

	[illegible]	[illegible]
[illegible]	[illegible]	
[illegible]	0.1 (0.87)	0.1 (0.5)
[illegible]	0.9 (0.87)	0.7 (0.67)
[illegible]	0.9 (0.32)	0.9 (0.82)

Key [translator's note: see original description for symbols, numbers and English]:

	Insides of Upper Arms	Part Between Cheek and Chin
Gel	Score of tightening effect	
Gel A		
Gel B		
Gel C		

[0033] According to Table 5 it was confirmed that, when the gels B and C were used, there was an actual feeling of a tightening effect on the insides of the upper arms and in the part between the cheek and chin.

[0034] <Practical Example 8>

An agent (gel) for ameliorating sagging of the skin and tightening the skin having the formulation shown below was manufactured in the usual method.

[0035] [Table 6]

(Ingredients)	(% by weight)
Polyacrylic acid (Carbopole, made by Goodrich Co.)	0.5
Calcium hydroxide	0.15
Glucam	10.0
86% glycerin	10.0
Glycine betaine	3.0
Rosemary extract (Practical Example 1)	1.0
Succinic acid	1.5
Purified water	Balance
Total	10.0

[0036] <Practical Example 9>

An agent (milky lotion) for ameliorating sagging of the skin and tightening the skin having the formulation shown below was manufactured in the usual method.

[0037] [Table 7]

(Ingredients)	(% by weight)
Palmitic acid	0.5
Olive oil	2.0
Cetanol	1.0
Jojoba oil	5.0
Sodium monohexadecyl phosphate	2.0
Sorbitan monostearate	0.5
Glycerin	15.0
Ethanol	5.0
Burdock root extract (Practical Example 5)	1.0
Kiwi extract (Practical Example 3)	0.5
Milky lotion	2.0
Purified water	Balance
Total	100.0

[0038] <Practical Example 10>

An agent (face lotion) for ameliorating sagging of the skin and tightening the skin having the formulation shown below was manufactured in the usual method.

[0039] [Table 8]

(Ingredients)	(% by weight)
Burdock root extract (Practical Example 6)	0.2
Carrot/ginseng extract (Practical Example 7)	0.2
86% glycerin	15.0
Dipropylene glycol	5.0
Purified water	Balance
Total	100.0

[0040] [Advantages of the Invention]

By using the agent for ameliorating sagging of the skin and tightening of the skin of the present invention, the skin can be provided with a tightened feeling.